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DNA Base Sequence Homology between Coliphages T7 and ϕII and between T3 and ϕII as determined by Heteroduplex Mapping in the Electron Microscope

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(*Received 15 January 1973, and in revised form 19 March 1973*)

The genetic relatedness of the similar coliphages T7, T3 and ϕII was investigated by electron microscopic observations of the DNA heteroduplexes formed with one DNA strand from coliphage T7 (or T3) and the complementary strand from ϕII. The T7–ϕII heteroduplex is almost invariant with denaturing conditions, below the melting temperature of T7 DNA. This observation indicates that the DNAs of T7 and ϕII have regions of complete homology and regions of complete non-homology. In direct contrast, the T3–ϕII heteroduplex varies with denaturing conditions. The fraction of the T3–ϕII heteroduplex observed as double-stranded decreases with an increase in denaturing conditions below the melting temperature. This observation indicates that the DNAs of T3 and ϕII have extensive sequences of partial homology.

1. Introduction

Davis & Hyman (1971) observed that the fraction of the coliphages T7–T3 heteroduplex appearing as double-stranded decreased with an increase in denaturing conditions, below the melting temperature (T_m) of T7 DNA. Simon *et al.* (1971) had observed in contrast that the λ–434 heteroduplex appeared invariant with denaturation conditions, over the same range. The heteroduplex derived from the two phages capable of extensive productive recombination, λ and 434, is composed of stretches of complete base sequence homology and stretches of complete non-homology. The heteroduplex derived from two related phages incapable of extensive productive recombination, T7 and T3, is composed of extended stretches of partial homology. The hypothesis derived from these observations was that the process of recombination in some way mediates selection for either complete base sequence homology or complete non-homology within discrete regions of the genome. Related phages which do not recombine are not subject to that selective pressure and therefore accumulate random base changes.

Seeking further experimental amplification of the hypothesis, we noted that T7 and its relative ϕII do undergo recombination (Linial, 1971). We predicted, observed, and report here that the T7–ϕII heteroduplex appears invariant with denaturing conditions, below the T_m of T7 DNA. The T3–ϕII heteroduplex is almost completely variant with denaturation conditions, showing less duplex as the T_m is approached.

2. Materials and Methods

The T7 strain used here was from Dr F. W. Studier, who had obtained it at Caltech from Dr R. L. Sinsheimer. The T7 strain used in the previous study (Davis & Hyman, 1971) was from the Caltech stocks of the late Dr J. J. Weigle. Both these strains are derived from the collection of Dr M. Delbrück. The heteroduplex composed of one strand of the Weigle stock and the other of the Studier stock appeared completely duplex when observed in the electron microscope (M. N. Simon, personal communication). The Studier stock has been used because of its extensive genetic characterization (Studier, 1969). Strain T3 was from the American Type Culture Collection, and is the same strain as used in the previous study (Davis & Hyman, 1971). Strain ϕII was from the collection of Dr M. Malamy, who obtained it from Dr K. B. Low, who received it from Dr E. Adelberg, who had obtained it at the Pasteur Institute in about 1959 (Linial & Malamy, 1970).

The procedures for separating strands of the various DNAs and for forming heteroduplexes (in this case, containing the T7 *l*-strand and the ϕII *r*-strand or the T3 *r*-strand and the ϕII *l*-strand) have been described previously (Davis & Hyman, 1971). The basic protein film technique was described by Davis *et al.* (1971). The T7–ϕII and the T3–ϕII heteroduplexes were mounted under four different isodenaturing formamide concentrations. The isodenaturing procedure has been described in detail before (Davis & Hyman, 1971).

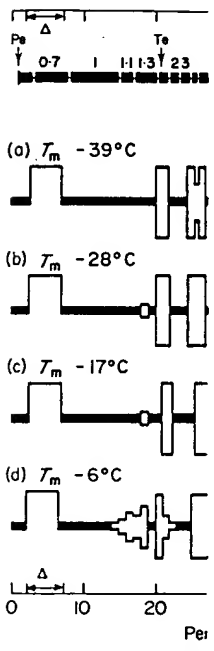
3. Results

A sample electron micrograph of the T7–ϕII heteroduplex (at T_m—28°C) is shown in Plate I. The T7–ϕII heteroduplex is almost entirely duplex, though a deletion loop close to one end followed shortly by two characteristic non-homology loops is seen (Plate I, Fig. 1). There are four to six small non-homology loops on each molecule. The two single strands of these loops appear about equal in length.

The deletion loop of the T7—ϕII heteroduplex can be used to position the heteroduplex left to right on the T7 physical map. The T7–ϕII heteroduplex is compared to the heteroduplex composed of ϕII and the DNA deletion mutant of T7, called T7H1 (Studier, 1972). T7H1 harbors a DNA deletion from 2·9% to 7·0% from the left end (M. N. Simon & F. W. Studier, personal communication). If the deletion loop of the T7–ϕII heteroduplex is near the right end, then the T7H1–ϕII heteroduplex will show deletion loops near both ends. If the T7–ϕII deletion loop is near the left end, then the T7H1–ϕII heteroduplex will be duplex on the right end and changed on the left end. When the T7H1–ϕII heteroduplex was examined, no deletion loop was seen; both ends were duplex. The two characteristic non-homology loops which were previously observed near the T7–ϕII deletion loop were still present. Therefore, the T7–ϕII deletion loop is on the left end. It can be concluded that this loop is formed because of a deletion in ϕII DNA relative to T7 DNA and that this deletion covers the region equivalent, within experimental error, to the H1 deletion of T7 DNA.

Confirmation of this assignment of the left end of the T7–ϕII heteroduplex comes from a comparison of the early RNAs of T7, T7H1 and ϕII. The early region of T7 extends from 1% to 20% from the left end (Hyman, 1971; Studier, 1972). The RNAs transcribed from this region by the *Escherichia coli* RNA polymerase form characteristic bands upon electrophoresis in polyacrylamide gels (Siegel & Summers, 1970). It is predicted that the pattern for ϕII early RNA will match that of T7H1 early RNA rather than the pattern of T7$^+$ early RNA. This prediction has been verified experimentally (Summers *et al.*, 1973; Brunovskis *et al.*, 1973).

The T7–ϕII heteroduplex maps are presented in Figure 1. Most of the features are invariant with formamide concentration: the deletion in ϕII DNA extending from



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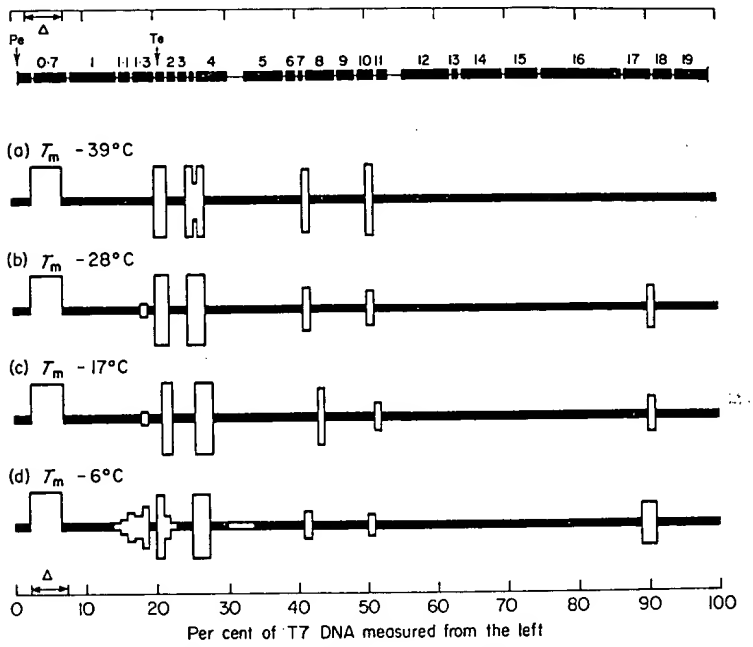

FIG. 1. T7-ϕII heteroduplex maps.

The T7-ϕII heteroduplex maps were constructed from measurements on heteroduplexes mounted under four different isodenaturing formamide concentrations. At each concentration, ten heteroduplex molecules were measured. For every molecule, ten molecules each of single-stranded and double-stranded ϕX174 DNA were measured in the same micrograph as length standards. Lengths of the single-stranded regions of the heteroduplex were first expressed as a function of single-stranded ϕX174 DNA, and duplex regions as a function of double-stranded ϕX174 DNA. The lengths were then converted relative to the length of T7 DNA (or T3 DNA, which is identical within experimental error) using the relationship that the ratio of the molecular lengths of ϕX174 DNA to T7 DNA (or T3 DNA) is 0·136 (Davis & Hyman, 1971). To construct the map of one heteroduplex molecule, its parts were added sequentially from one end. Solid thick regions represent duplex. Two thin opposing lines represent two single strands. Regions were occasionally found to be duplex in one heteroduplex but single-stranded in another, on the same grid. These regions are represented by two opposing lines close together. The fraction of the ten molecules observed to be single-stranded in a given region is directly proportional to the distance between the two opposing lines representing the region. The distance apart for observing all ten heteroduplexes as single-stranded in that region is seen in (b) from 20% to 22%. The deletion in ϕII DNA is represented by one thin line and by the symbol *delta* (Δ) at the appropriate position. The lengths of the single strands of a non-homology loop were averaged. The mounting conditions were as follows: (a) T_m −39°C, 30% formamide onto 5% formamide; (b) T_m −28°C, 45% formamide onto 15% formamide; (c) T_m −17°C, 60% formamide onto 30% formamide; (d) T_m −6°C, 75% formamide onto 45% formamide. The physical map of T7 at the top of the Figure was constructed as described by Davis & Hyman (1971) with the addition of the information provided by Studier (1972), Hyman & Summers (1972), Summers *et al.* (1973), and Simon & Studier (personal communication). Pe is the early promoter region (Davis & Hyman, 1970); Te is the site of strong termination at the right end of the early region (Hyman, 1971; Studier, 1972). The spaces between genes are for ease of visualization.

about 3% to 7% (per cent of T7 DNA measured from the left end); the non-homology loops between 20% and 21·5%, between 24·5% and 27% (though there is a slight tendency for part of that region to form base pairs at T_m—39°C), between 41% and 42%, and between 50% and 51%, the long stretch of duplex between 51% and 89%. The few changes in the heteroduplex as a function of formamide include the region between 90% and 91%, duplex at T_m—39°C, but single-stranded at T_m—28°C. At T_m—6°C, there is an additional melting between 89% and 90%. The regions between 14% and 19%, between 21·5% and 23%, and between 30% and 34% partially melt at T_m—6°C.

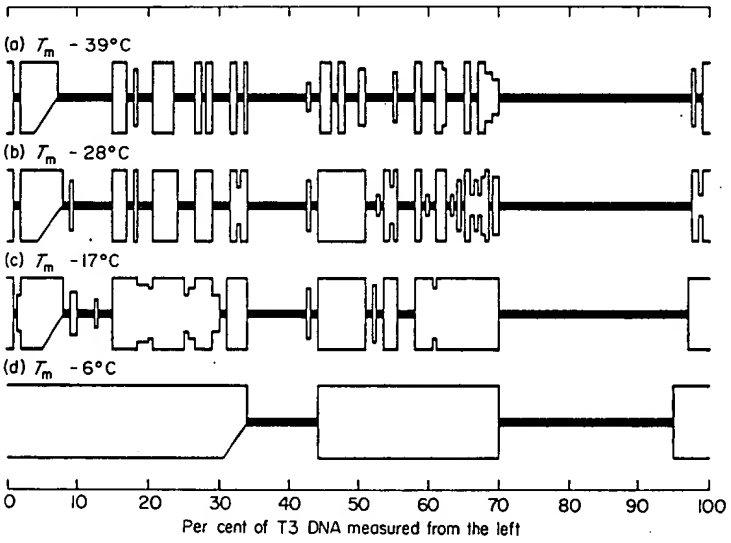

FIG. 2. T3–ϕII heteroduplex maps.

The T3–ϕII heteroduplex maps were constructed from measurements on heteroduplexes mounted under four different isodenaturing formamide concentrations as described in the legend to Fig. 1. For the T3–ϕII heteroduplex molecules, variation arises from two sources: random error and partial melting. Regions that correctly appear duplex on one molecule and single-stranded on another are sometimes difficult to recognize. The method used to minimize the random error is to locate and align the invariant regions of the T3–ϕII heteroduplex and then to normalize the regions in between. The distance apart for observing all ten heteroduplexes as single-stranded in that region is seen in (d). The deletion in ϕII DNA is represented by showing the appropriate non-homology loop as being composed of single strands of different length. The lengths of the single DNA strands composing the other non-homology loops have been averaged. There were occasionally features too small to represent graphically, particularly in a region mounted near its T_m, as 65% to 70% in (b).

A sample electron micrograph of the T3–ϕII heteroduplex (at T_m—39°C) is shown in Plate II. The DNA deletion in ϕII was used to identify the left end of the T3–ϕII heteroduplexes. The T3–ϕII heteroduplex maps are presented in Figure 2. In direct contrast to the homology between T7 and ϕII DNAs, the extent of duplex in the T3–ϕII heteroduplex decreases with increasing formamide concentration. There is an extended stretch of strong homology, double-stranded at T_m—6°C, near the right end of the heteroduplex. The terminally redundant ends are sufficiently non-homologous to remain single-stranded at T_m—39°C.

From the data of Figures 1 and 2, the overall percentage of duplex DNA *versus* formamide concentration can be plotted for both heteroduplexes, as well as T7 DNA

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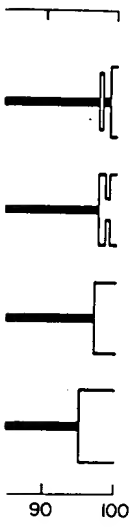

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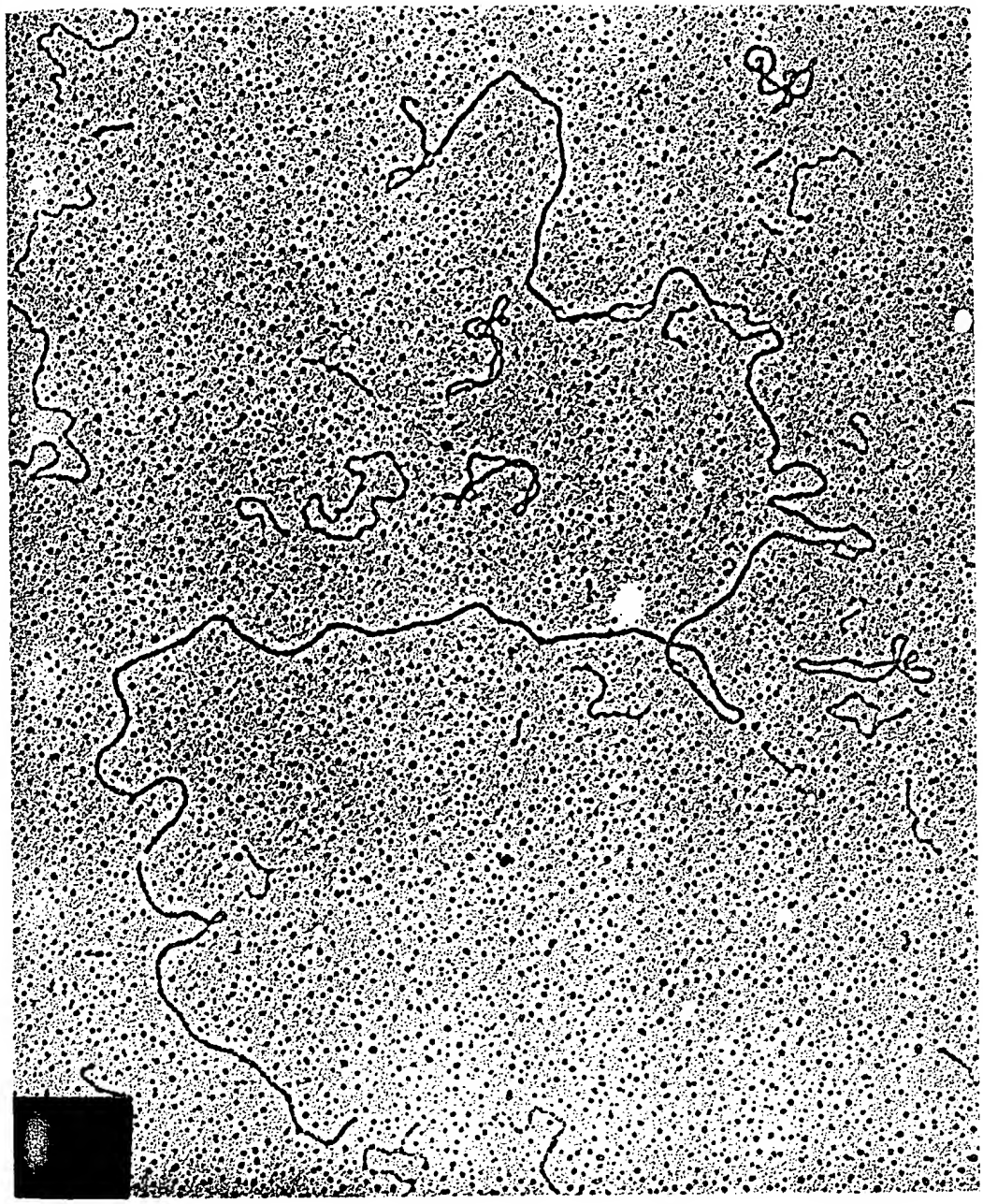

PLATE I. T7–ϕII heteroduplex at T_m −28°C.

The T7–ϕII heteroduplex was mounted from a 45% formamide solution (cation concentration 0·060 M) and layered onto a 15% formamide solution (cation concentration 0·0060 M). The temperature of mounting was 23°C. The left end of the molecule, near the deletion loop, is in the upper portion of the micrograph. The small circular DNAs are ϕX174. The grid was shadowed with Pt–Pd (80% to 20%).

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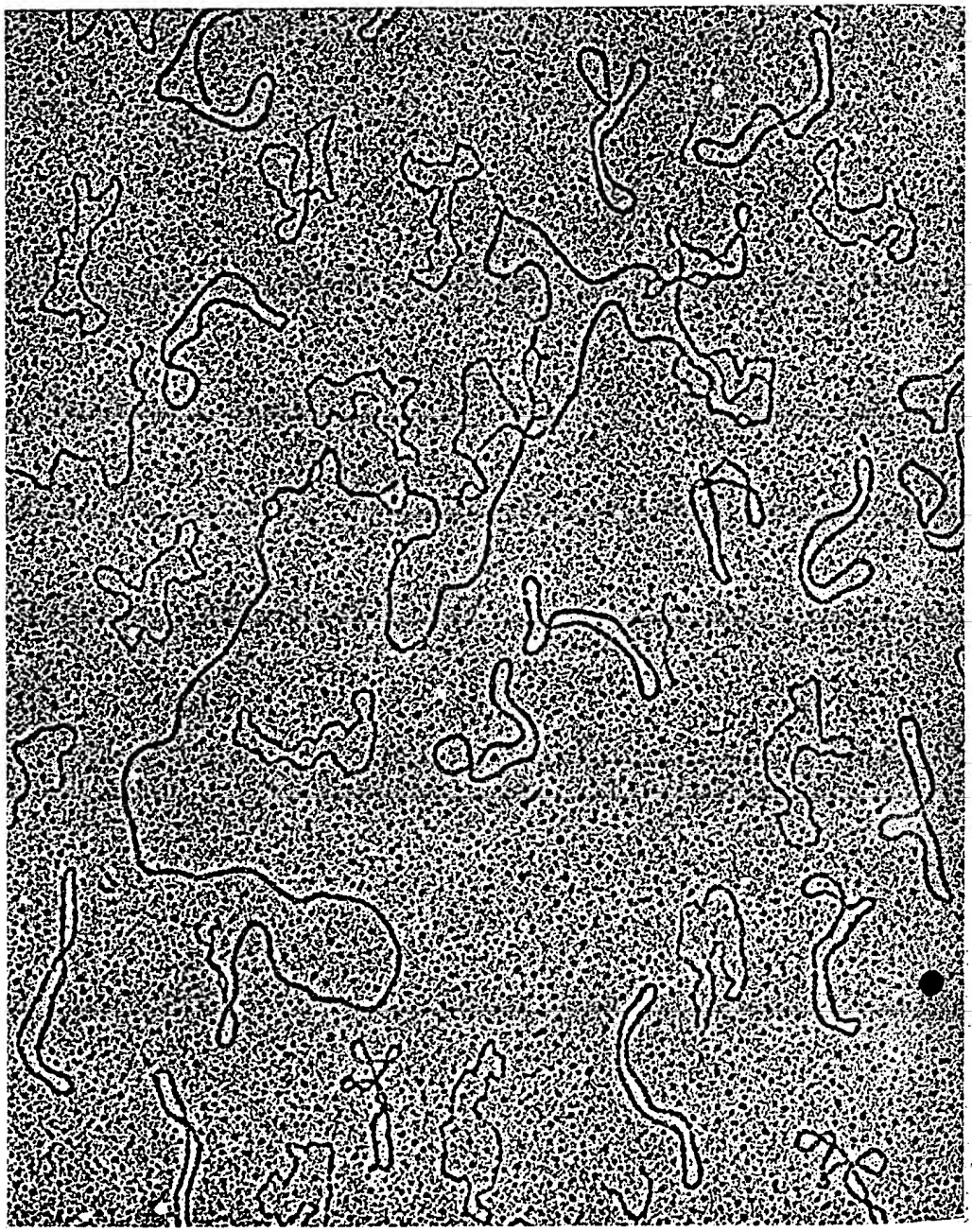

PLATE II. T3–ϕII heteroduplex at T_m −39°C.

The T3–ϕII heteroduplex was mounted from a 30% formamide solution (cation concentration 0·060 M) and layered onto a 5% formamide solution (cation concentration 0·0060 M). The temperature of mounting was 23°C. The left end of the molecule, near the non-homology loop composed of single strands of unequal lengths, is in the upper portion of the micrograph. The small circular DNAs are ϕX174. In this micrograph, the difference between the smooth, even duplex ϕX174 DNA and the uneven, "kinky" single-stranded ϕX174 DNA is particularly clear. The grid was shadowed with Pt–Pd (80% to 20%).

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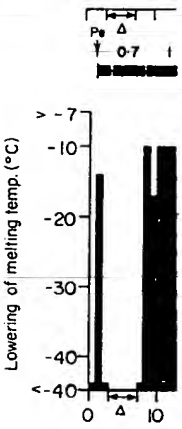

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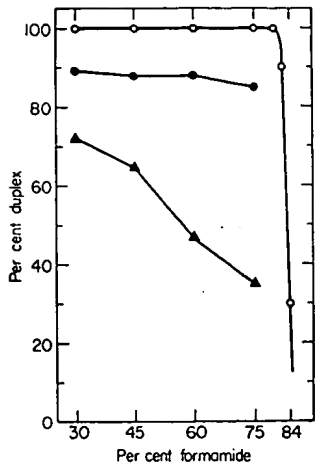

FIG. 3. Melting curves.

The percentage of T7 DNA, the T7–ϕII heteroduplex and the T3–ϕII heteroduplex appearing as duplex at each iso-denaturing formamide concentration is plotted against that formamide concentration. The data for the T7–ϕII heteroduplex are from Fig. 1 and for the T3–ϕII heteroduplex from Fig. 2. The data for T7 DNA are from Davis & Hyman (1971). Included as duplex are those regions appearing duplex in some heteroduplexes and single-stranded in others on the same grid, proportional to the fraction appearing as duplex. —○—○—, T7 DNA; —●—●—, T7–ϕII heteroduplex; —▲—▲—, T3–ϕII heteroduplex.

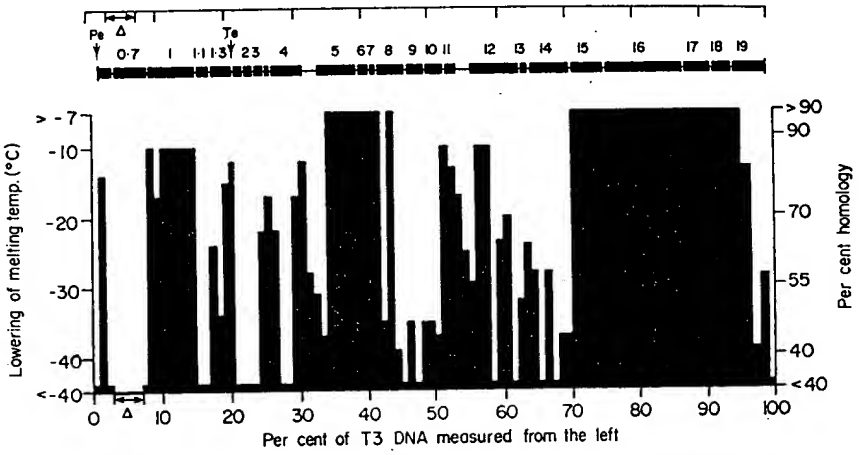

FIG. 4. T3–ϕII sequence homology map.

The T3–ϕII heteroduplex maps of Fig. 2 were arbitrarily divided into 1% intervals. A melting curve was constructed for each 1% interval, and the formamide melting concentration was determined. The lowering of the melting temperature relative to T7 DNA was calculated using the relationship that 1% formamide lowers T_m by 0·7 deg. C (McConaughy *et al.*, 1969) and is the left vertical co-ordinate. The actual percentage DNA base sequence homology was calculated for each 1% interval of the map using the relationship that 1 deg. C lowering of T_m corresponds to 1·5% base sequence mismatch in poly(dA-dT) (Laird *et al.*, 1969) and is the right vertical co-ordinate. The deletion in ϕII DNA is represented at the appropriate position by the symbol *delta* (Δ). The construction of the physical map of T7 is described in the legend to Fig. 1, and is used here by analogy. Pe, early promoter region; Te, site of strong termination at the right end of the early region.

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as a control. Figure 3 presents the melting curves. The fraction of duplex of the T7–ϕII heteroduplex is 88% duplex, with only a slight increase at T_m—39°C, and a small decrease at T_m—6°C. Linial *et al.* (1970) have previously shown that T7 RNA hybridizes efficiently with ϕII DNA and *vice versa*. The amount of duplex of the T3–ϕII heteroduplex decreases continually with increasing formamide concentration; 72% duplex at T_m—39°C to 35% duplex at T_m—6°C. This melting at formamide concentrations where T7 DNA appears completely duplex defines regions of partial homology.

The melting curve for the T3–ϕII heteroduplex (Fig. 3) gives the over-all average percentage of duplex. However, from the data of Figure 2, the melting of each 1% length interval (arbitrarily chosen) of the heteroduplex can be considered separately to obtain a detailed map of per cent homology between T3 and ϕII (Fig. 4).

4. Discussion

While ϕII DNA is generally homologous to T7 DNA, in the regions between 41% and 42% and between 90% and 91% (gene *17*, a tail protein), ϕII DNA is homologous to T3 DNA (Figs 1 and 4). The assignment of gene to function for T7 is from Studier (1972). In the region between 24·5% and 27% which includes part of gene *3* (endonuclease), gene *3.5*(lysozyme) and part of gene *4* (involved in DNA synthesis), ϕII DNA is not homologous to T7 DNA, but is about 68% homologous to T3 DNA. In the region between 50% and 51% (gene *10*, the major head protein), ϕII is not homologous to T7 and is not more than 43% homologous to T3. In the region between 20·5% and 22% (gene *1.7*, a dispensable gene of unknown function), ϕII is neither homologous to T7 nor to T3. The deletion in ϕII DNA occurs in the region equivalent to gene *0.7* in T7 DNA. T7 gene *0.7* codes for a protein involved in the rapid shut-off of host transcription (Brunovskis & Summers, 1972). Linial & Malamy (1970) earlier demonstrated that ϕII infection did not result in rapid host shut-off. The explanation is now obvious: ϕII DNA is deleted in that gene.

The strongest DNA homologies among T7, T3 and ϕII are between 70% and 89%. This region includes genes *15* and *16* (both minor head components) and gene *17* (a tail protein). Apparently, alterations in the amino acid sequences of these proteins are not well tolerated.

T7 DNA, T3 DNA, and ϕII DNA have terminal redundancies extending about 0·7% (Ritchie *et al.*, 1967). The terminal redundancy of ϕII is homologous to that of T7, but is not homologous to that of T3. The function of the terminal redundancy is not known, though the recent suggestion of Watson (1972) that it plays an essential role in DNA replication is attractive. If the terminal redundancy of the DNA is necessary for the survival of the phage, and if two phages have non-homologous terminal redundancies, as do T3 and ϕII or T7 and T3, the DNA products of a single recombination would not be terminally redundant and, therefore, presumably would not be viable. Evolutionary pressure would select for phages that have evolved an exclusion mechanism to prevent the production of lethal recombinants after simultaneous infection. T7 and T3 are known to exclude one another (Hausmann *et al.*, 1961). An exclusion mechanism assures that phage with non-homologous terminal redundancies are not capable of extensive productive recombination. Their DNAs will accumulate random base sequence changes.

On the other hand, for phages such as T7 and ϕII, recombination will occur in regions of sufficient DNA homology and the "best" sequence for a gene will be adopted for all the phage. This process will maintain complete homology within those regions. In regions of insufficient homology, recombination will not occur.

An additional possibility for the evolution of a region of complete non-homology is that one phage DNA has picked up a section of host DNA. This possibility is more attractive for lysogenic phages such as λ than for virulent phages such as T7, which degrade the host DNA after infection.

These results support the interpretation of evolution and subsequent selection put forward by Simon *et al.* (1971) and Davis & Hyman (1971), and in addition suggest evolutionary patterns for T7, T3 and ϕII which may be in part a consequence of the mode of replication of these phages.

We thank Mrs Joan Niles and Mr Clifford Scheiner for their measurements of the electron micrographs. Drs F. W. Studier and M. N. Simon generously provided us with their data before publication. This work was supported by United States Public Health Services grant CA06519, and the American Cancer Society grant VC60A.

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Note added in proof. McConaughy *et al.* (1969) found that 1% formamide reduced the T_m by 0·7°C. Recently, Blüthmann *et al.* (1973) reported that 1% formamide reduced the T_m by 0·6°C. Wetmur & Davidson (1968) measured the T_m of T7 DNA in the absence of formamide to be 98°C at a Na^+ concentration of 1·0 M. Record & Zimm (1972) measured the T_m of T7 DNA to be 33·8°C in 89% formamide at a Na^+ concentration of 0·3 M and 31·2°C in 80% formamide at a Na^+ concentration of 0·08 M. The equation presented by Frank-Kamenetskii (1971) relating T_m to base composition and ionic strength can be used to calculate that in the absence of formamide the T_m of T7 DNA is 99°C at a Na^+ concentration of 1·0 M, 92°C at a Na^+ concentration of 0·3 M and 83°C at a Na^+

concentration of 0·08 M. The agreement between the experimental and calculated values for the Na^+ concentration of 1·0 M adds credence to the other two values. Calculations based on these latter data yield the relationship that 1% formamide lowers the T_m of T7 DNA by 0·65°C, a number between the values reported by McConaughy *et al.* (1969) and by Blüthmann *et al.* (1973). Thus, all the data are in reasonable agreement.

Laird *et al.* (1969) found that the T_m was reduced by 0·7°C for each per cent mismatch in acid deaminated poly [d(A-T)], a purine–pyrimidine mismatch. Recently, Ullman & McCarthy (1973) found the T_m was reduced by 2·2°C for each per cent mismatch in alkaline deaminated DNA, another type of purine–pyrimidine mismatch. Hutton & Wetmur (1973) reported that the T_m was reduced by 1·1°C for each per cent mismatch in glyoxylated DNA, which is a model for purine–purine mismatch. From these numbers, it seems reasonable at present to take as an "average" value that the T_m is reduced by 1·4°C for each per cent mismatch. From this correlation, a region that appears double-stranded at T_m −6°C has less than one base pair mismatched in 25. A region that appears single-stranded at T_m −39°C has at least one base pair mismatched in three.

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